SCIENCE

FRIDAY, AUGUST 4, 1916

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THE METHOD OF GROWTH OF THE LYMPHATIC SYSTEM[1]

IN selecting a title connected with the general subject of the lymphatic system, I have chosen to emphasize the phase of the subject with which the anatomist of to-day is concerned. As a matter of fact, in studying the problem of growth he is seeking to understand the nature of the lymphatic capillary. This is no new problem, but rather it has dominated the study of the lymphatic system for nearly three hundred years. The colorless fluid of the tissues was called lymph long before lymphatics were discovered. It was thus natural that when vessels were discovered containing this fluid they were called lymphatics. As soon as the lacteals and then the general lymphatics were discovered, the question arose in regard to the nature of these vessels, what was their extent and how they ended in relation to the surrounding tissues. At first the lymphatics were thought to begin in wide mouths in the walls of the various cavities of the body, and then, as these openings proved difficult to find, attention became focused on the relation of the lymphatics to the tissues. The number of terms which have been used in seeking to analyze the relation of the lymphatics to the tissues—for example lymph radicles, lymph rootlets, lymph spaces, parenchymal spaces, tissue spaces—will serve to illustrate how persistent has been the quest of the anatomist to understand the lymphatic capillary. Stated in other terms, this is the time-honored question of open and closed lymphatics. In presenting to you the conception

[1] Address delivered to the Harvey Society of New York City on December 18, 1915.

of lymphatic capillaries as definite vessels completely lined by endothelium, and related to tissue-spaces just as blood-capillaries are, it will be necessary to emphasize first the importance of tissue-spaces. Indeed, the general subject of tissue-spaces, as important systems in the body, related to blood-capillaries and to lymphatic capillaries in function, is, I believe, nowhere sufficiently emphasized in the literature.

It is well known that the plasma of the blood is constantly exuded from the blood-vessels into the tissue-spaces, so that all the cells of the supporting tissues, as well as the special cells of each organ, are bathed in fluid. Moreover, it is obvious that with all the varying activities of the cells of the body, the fluid becomes laden with different nutritive and with different stimulative substances and with different waste products, so that it varies widely in its composition. The subject of tissue-spaces—meaning not empty spaces, but spaces which always contain fluid—is by no means a simple one. There are primarily the general, small spaces to which I have just referred, between all of the fibers and cells of the connective tissues and between the parenchyma of each organ and its supporting tissues: but there are also special systems of great spaces, which arise from the small spaces by a definite method, which have a definite structure and contain a fluid which is different from the other fluids in the body—such, for example, as the subarachnoid spaces which surround the central nervous system.

That the cerebro-spinal fluid is secreted by a special organ and contains certain products of internal secretion is now known. The pia-arachnoid membrane has been shown by Weed[2] to have an extremely interesting structure and development. I will mention here only the very important arachnoidal villi which are lacelike projections of the arachnoid into the dura. They lie along the dural veins and lead to the dural sinuses. These villi, which he has shown to be the main organs of absorption for the cerebro-spinal fluid, are covered with a layer of mesothelial cells, which tend to become more abundant at the tips, forming cell nests.

Other great systems of spaces are found in the internal ear and in the eye. The scala tympani and scala vestibuli of the cochlea have been called peri-lymphatic spaces, though they have no relation to the lymphatic system. These spaces of the ear have just been shown by Streeter[3] to have a most interesting development. The scala tympani and scala vestibuli are formed from spaces in the mesenchyme which at first become slightly larger than the usual spaces and then coalesce into still larger spaces. Moreover, this process is not indefinite, but has two distinct places of origin, one between the sacule and the oval window and the other between the cochlea and the round window. From these two areas the formation of the two great spaces of the cochlea proceeds in a definite and constant direction, so that a model of their form from one specimen is the same as that from any other specimen of the same stage. Moreover, when studied in sections this process appears to be a gradual dilatation of preexisting tissue-spaces, with a disappearance of more and more of the original connective tissue syncytium, rather than being caused by a differentiation of the mesenchyme cells forming the border of these spaces. As the cavity thus formed reaches its ultimate dimensions some of the remaining mesenchyme cells do differentiate to form a mesothelial lining. I emphasize this method of the formation of a cavity out of mesenchymal spaces, for the

2 Weed, L. H., *Jour. of Med. Research*, Vol. 31, 1914.

3 Streeter, G. L., to be published in the "Proc. of the Amer. Asso. of Anat.," 1916.

reason that I believe it to be essentially different from the method of formation of blood-vessels.

Again in the eye there are two cavities having an entirely different development. Posterior to the lens is a space filled with fluid, which begins not by a hollowing out of tissue-spaces in mesenchyme, but as a definite differentiation of a primitive vitreous body by the retina. In the formation of this body the mesenchyme is only secondarily concerned. On the other hand, the history of the aqueous chamber of the eye is analogous to that of the formation of the cerebro-spinal system of tissue-spaces.

Along the pathway of the blood-vessels of the central nervous system are special chains of tissue-spaces, lined by an indefinite mesothelium, but arranged in sufficiently definite lines to have received the name of peri-vascular lymphatic spaces. These spaces, however, have no relation to lymphatics and should be called perivascular tissue-spaces. Along the nerves also are chains of spaces which can be injected in the embryo, and which may be termed perineural spaces. Similar chains of connecting spaces have been injected by Lhamon[4] along the course of the Purkinje fibers of the heart. Besides these very interesting special systems of tissue-spaces there is a group of great spaces which is still better known—namely, the great serous cavities of the body. These cavities, which form as a dilatation of spaces in the mesenchyme, have also a definite embryological history, a definite cellular wall of mesothelium, and a special very scanty content of fluid.

In order to analyze the relation of the general tissue-spaces and of these special systems of large tissue-spaces which develop out of the general ones, it is necessary to submit them all to some type of experiment. Fluids containing a suspension of minute granules or true solutions whose location can be detected subsequently by the precipitation of granules injected into these various spaces give widely and astonishingly different results. Weed has carried out a very interesting series of experiments of injections into the subdural and subarachnoid spaces. In these experiments he injected a solution of potassium ferrocyanide and iron ammonium citrate, at the same time withdrawing an equivalent amount of cerebro-spinal fluid, to eliminate phenomena due to pressure. He found that when the granules of Prussian blue were precipitated by an acid-fixing agent, they were in the meshes of the arachnoidal villi, within the cells of the nests of mesothelium at their tips and within the dural sinuses. On the other hand, when he produced a cerebral anemia by bleeding, the fluid was sucked into the special and very important tissue-spaces that surround the nerve-cells. These experiments demonstrate conclusively that the central nervous system has a special system of tissue-spaces beginning, one might say, with the spaces surrounding every individual nerve-cell of the brain, extending into the subarachnoid area and draining not by lymphatics, but by another special system of absorbents—namely, the arachnoidal villi—into the cerebral sinuses. Wegefarth[5] has shown that the anterior chamber of the eye has a similar system of absorbents, the pectinate villi. These lead to the canal of Schlemn, a vein analogous to the cerebral sinuses.

When injections are made into the peritoneal cavity the results vary widely, according to the nature of the fluid injected. As a matter of fact our knowledge of this important subject is far from complete, but it has been shown that certain true solutions are absorbed by the blood-vessels. On

[4] Lhamon, R. M., *Amer. Jour. of Anat.*, Vol. 13, 1912.

[5] Wegefarth, P., *Jour. of Med. Research,* Vol. 31, 1914.

the other hand, it is known that granules are in large part taken up by special large phagocytic cells, some of which pass into the lymphatics of the diaphragm. This gives a suggestion of a possible differentiation in absorption between blood-vessels and lymphatics. Indeed, a partial differentiation in function is a most familiar phenomenon: I refer to the villi of the intestine, where almost all of the fat passes into the central lacteal while the carbohydrates pass directly into the blood-stream. It is well known, on the other hand, that when a needle is introduced into certain areas under the skin or into specific layers of many of the organs and a fluid containing granules is injected, the granules always appear in the lymphatic trunks which drain the area. What is the difference between tissue-spaces which are drained by lymphatics and those which are not? What is the difference between areas in which injections always show lymphatics and those which never show lymphatics? What is the nature of the fluids which pass through the lymphatics and those which do not? In other words, exactly what happens at the point of the needle when an artificial edema is produced? This I understand to be the meaning of the main problem connected with the lymphatic system—the solution of the enigma of the mechanism of absorption. The difficulty of the problem was well expressed by Bartels[6] as late as 1909, when he said that the relation of the lymphatic capillary to the tissue-spaces was a philosophical rather than an anatomical problem. My understanding of the recent work on the lymphatic system is that it tends to take the system out of the realm of the mythical and to make it a definite anatomical entity. The investigations of the last fifteen years have demonstrated that the blood-vessels are the primary absorbents, and that subsequently partial systems of absorbents develop, such as the arachnoidal villi and the lymphatics which drain into the veins.

I have been greatly interested in the attempts of the earlier anatomists to solve the problem of absorption. They brought to the subject of tissue-spaces and the fluid within them a great freshness of interest and constantly sought to understand the meaning of their various observations. They saw the arteries become smaller and smaller, they were familiar with lymphatic trunks and with some lymphatic capillaries. What then was more natural than to assume that when the arterioles became so small that the corpuscles could not enter, there were still smaller vessels which carried the plasma over into the lymphatics? These tiny hypothetical vessels were called "vasa serosa." A belief in their existence was held throughout the eighteenth century, and was not overthrown until the discovery of cells by Schwann in 1830. Schwann believed that the mesenchymal cells were hollow and from this idea Virchow formulated the theory that hollow connective-tissue cells spanned the gap between the blood-vessels and the lymphatics. Then followed the discovery by von Recklinghausen that the wall of the lymphatic capillary was composed of cells. Von Recklinghausen thought that silver impregnations showed that lymphatics spread out as lymph radicles or lymph rootlets into the tissue-spaces. At first His believed in these lymph radicles, that is, in open lymphatics, but von Recklinghausen's discovery of endothelium led him to a conception of a lymphatic capillary as a definite, closed vessel, this conception being confirmed by his own experience with injections. If lymphatics open out into tissue spaces every injection of a capillary plexus with a non-

[6] Bartels, P., "Das Lymphgefässystem. Handbuch der Anatomie des Menschen," Von Bardeleben, 1909.

diffusible fluid should spread out into tissue spaces and obscure the vessel—which is most obviously not the case. Thus von Recklinghausen's discovery served to bring up anew the question of open and closed lymphatics.

During the present century it has become evident that some light might be thrown on the obscure question of the relation of tissue spaces to lymphatic capillaries through the study of their development. The first general hypothesis concerning the origin of the lymphatic system in the embryo was that fluid exuded from the peripheral blood-vessels and gradually hollowed out channels. As the fluid increased, these vague channels were thought to extend from the periphery to the center and then establish connections with certain veins. This hypothesis was made concrete by Gulland,[7] who found large empty vessels in the skin of embryos about 4 cm. in length, which he thought to be the first lymphatics. In reality the lymphatics begin much earlier. This general hypothesis was to some extent modified by studies of Budge[8] and Sala.[9] Budge injected the extra-embryonal celom in early chick-embryos, and got patterns of injection in the area vasculosa vaguely simulating lymphatics. These patterns we now know were produced by fluid passing out of the celom into the network of spaces between the plexus of blood-capillaries. Budge then made beautiful injections of true lymphatics in much later stages, and to explain his observations built up the hypothesis that there was a primitive lymphatic system associated with the body cavity and a later, secondary system of definite ducts. The thoracic duct he believed formed the connection between these two systems. These observations of Budge, which we now know to be incorrect, are, however, of great interest to the embryologist—representing as they do the earliest groping in darkness in hope of finding the first lymphatics. The work deserves emphasis also as the only basis of all the erroneous theories surrounding the idea that the body cavity is in some especial way a part of the lymphatic system.

Another very interesting attempt to find the first lymphatics is shown in the work of Sala, who studied the origin of the posterior lymph-hearts in the chick. We know now that these lymph-hearts arise as endothelial buds from the walls of the coccygeal veins and that these buds develop into a plexus, which becomes a pulsating lymph-heart. Sala, working with this rapidly developing plexus, somewhat vaguely appreciated its relation to the veins: he described a hollowing out of cavities in the mesenchyme near the veins and then said that in the last analysis these cavities in the mesenchyme were from their first appearance nothing but terminal dilatations of the veins. However, he concluded that the lymphatics begin as excavations in the mesenchyme which soon join the veins. The confusion in Sala's description is now easily understood. Dominated by the theory that lymphatics were tissue-spaces, he could not analyze the evidence that they were from the start connected with the veins, and so described them as both veins and tissue-spaces. He made it clear, however, that he believed that the ducts were formed from chains of tissue-spaces hollowed out in the mesenchyme and lined by flattened-out cells. Sala's work, however, places the first lymphatics close to the veins, and demonstrates the difficulties of relying on the interpretation of sections in unraveling problems of growth.

Sala's work was published in 1900, and

[7] Gulland, *Jour. of Path. and Bact.*, Vol. 2, 1894.

[8] Budge, A., *Arch. f. Anat. u. Phys.*, Anat. Abth., 1887.

[9] Sala, L., *Ricerche Lab. di Anat. Norm. d. r. Univ. di Roma*, Vol. 7, 1899–1900.

during that year I was working on the development of the lymphatic system.[10] I began the investigation by injecting the foot-pads of young pig embryos. This procedure never fails to demonstrate lymphatics in the adult, and the same is true of fetal stages, but it was soon found that in embryos less than 3 cm. in length it was necessary to introduce the needle nearer the central veins in order to find lymphatics. By a long series of such injections the fact was gradually established that the skin of the embryo is invaded by lymphatics from two general regions—the neck and the groin. By noting the lines of growth of these invading vessels it was possible to obtain injections, showing the extent of the invasion of the skin for each stage. Moreover, in making these injections into the translucent skin of the embryo it became evident that in order to fill the lymphatics the needle must be introduced at a very exact level. When the needle cuts the lymphatics, the vessels can be seen to fill up from the oblique opening of the needle, without any extravasation if the pressure is light. If the needle is entered too superficially a bleb is always formed: if too deeply, the injection mass spreads out in straight lines, very characteristic and very different from lymphatics. These observations emphasize the lymphatic capillary as a definite vessel located at a specific level. Through a long series of such injections these definite lymphatic vessels were traced back to tiny buds close to the veins. The theory was then advanced that the entire lymphatic system consists of definite vessels of endothelium, which grow as blind buds from the endothelium of the veins and partially invade the body. The theory throws the emphasis on endothelium as the essential tissue of the lymphatic system, and premises that the endothelium of the lymphatic system is derived from the endothelium of the veins. This means that lymphatic vessels arise as an active growth of endothelial cells and are not formed by a passive dilatation of spaces. The outgrowth theory has not been established without opposition. There has been, indeed, a vigorous effort in this country to re-establish the older hypothesis of the origin of lymphatics from tissue-spaces, but in my judgment these efforts have not been successful.

I shall now outline briefly certain facts which have been established concerning the development of the lymphatic system. The lymphatic system begins in the human embryo of about 10 mm. in length—that is, during the sixth week of development. The first lymphatics are blunt buds which come from the internal jugular veins at the root of the neck. They are filled with blood which backs into them from the vein. These buds soon establish connections with each other and form a plexus which develops into a large sac, having its base on the internal jugular vein and arching into the posterior triangle of the neck. From this sac, which is astonishingly large, lymphatics grow out to the skin of the head and neck, to the thorax and arm, and partially invade the deep structures of the head. From the portion of the sac in the posterior triangle of the neck, vessels grow forward and form an extensive plexus along the external jugular vein. The knowledge of the form of this sac, of its position with reference to the internal jugular vein, and the pattern of the plexuses which develop from it, has unraveled the complicated and puzzling relations of the lymphatic ducts to the chains of lymph glands in the neck. The sac itself is transformed into different groups of lymph glands which might be analyzed as the primary lymph glands of

[10] Sabin, F. R., Johns Hopkins Hospital Reports, Monographs, New Series, No. 5, 1913. Gives a list of the literature.

the neck, and these primary lymph glands bear a definite relation to the secondary glands which form along the ducts growing out from the sac.

At a slightly later stage—in embryos of the seventh week, approximately 20 mm. in length—a series of lymphatic buds develop from some of the abdominal veins. These early buds have proved more difficult to study than the jugular buds—first because the veins from which they arise are more complex and were less well known, and secondly because their deep position has made direct observation in the living embryo and direct, precise injections practically impossible. Therefore our knowledge of the extent and origin of the abdominal lymphatics from different veins is still far from complete. Certain very interesting observations by Silvester[11] on monkeys and by Job[12] on rats show that in these forms certain lymphatic ducts drain permanently into the inferior vena cava, the iliac, the renal or the portal veins, suggesting a multiple origin of lymphatics from the abdominal veins. The main abdominal lymphatics begin as a retroperitoneal sac which develops from a vein connecting the two Wolffian bodies. This vein ultimately forms a part of the inferior vena cava. This large retroperitoneal sac furnishes the key for the study of the abdominal lymphatics. The lymphatics of the skin of abdomen and for the legs grow from paired iliac sacs. The retroperitoneal sac and the paired iliac sacs become connected with the left jugular sac by means of the thoracic duct, which grows from the left jugular sac and from the abdominal lymphatics, and is complete in embryos about 25 mm. long. There is thus formed a primary lymphatic system of sacs connected by the thoracic duct; this system in most mammals drains into the internal jugular veins on either side. From the primary sacs, a plexus of capillaries invades the body. In a general way, the vessels from the jugular sacs grow to the head, thorax and thoracic viscera; those from the retroperitoneal sac to the abdominal viscera, and in part to the thoracic viscera; and those from the iliac sacs to the abdominal walls and legs.

The injection of these invading plexuses of lymphatics from the sacs outward is possible in the embryo, though it is impossible in the adult, owing to the fact that the early vessels are without valves. In a general way it may be stated that by the time a fetus has reached the length of 5 cm. almost the entire skin has been invaded by a single plexus of lymphatic capillaries and the organs have received their primary lymphatic vessels. At this stage of embryonic development injections of any part of the lymphatic plexus spread out in all directions, so that theoretically the injection of any capillary might fill the entire system. I have injected the thoracic duct, for example, from the skin of the thorax, the injection mass passing around through the iliac lymphatics; or again I have injected the lymphatics of the skin by puncturing the thoracic duct. This complete anastomosis of the primary lymphatic capillary plexus of both the superficial and the deep systems in the embryo seems to me to be of considerable importance.

To illustrate the development of the lymphatic system to an organ and without an organ, I shall describe Cunningham's[13] work on the lymphatics of the lung. He has found that lymphatics approach the lung from three sources—from the two jugular sacs there are right and left lymphatic trunks and from the retroperitoneal sac

[11] Silvester, C. F., *Amer. Jour. of Anat.*, Vol. 12, 1911–12.

[12] Job, T. T., *Anat. Record*, Vol. 9, 1915.

[13] Cunningham, R. S., "Proc. Amer. Asso. of Anat.," *Anat. Record*, Vol. 9, 1915.

there are vessels which come up behind the diaphragm. The ducts which grow down from the neck meet in a plexus which surrounds the trachea. In the primitive lung, the general pattern of the organ is simple; it is obviously blocked off into large lobules by wide connective tissue septa. In the center of each lobule are the bronchus and the artery, in the septa are the veins. At the hilum the tracheal lymphatics divide into three plexuses, one spreading on to the pleura, a second following the bronchi and arteries, and the third the veins. The plexus which follows the veins grows rapidly to the pleura and spreads around the border of each primitive lobule, blocking off the pleura into polygonal areas. From this pattern the pleural lymphatics develop. The pleura is blocked off into its polygonal areas by the lymphatics when the embryo is about 5 cm. in length. At a much later stage, when the bronchi begin to develop atria and air sacs at their tips, the lymphatics grow down the center of the lobule along the bronchi. Just where the atria begin, the lymphatics turn sharply from the bronchi and pass out to the septa, so that the walls of the air sacs are without lymphatics.

The lymphatics of the diaphragmatic surface of the pleura grow up behind the diaphragm from the retroperitoneal sac, and injections of this surface of the lung in later stages fill up the pre-aortic, abdominal lymph glands. This relation of the pleural lymphatics to the abdominal lymphatics I believe to be of importance.

The development of the ducts to the intestines, and their differentiation within the intestinal wall into the ultimate lacteals of the villi, have also been worked out. The method of injection in the embryo affords an excellent opportunity to test the present belief in the partial invasion of organs by lymphatic vessels. For example, lymphatics have not been demonstrated in the adult liver beyond the capsule and the connective tissue septa, nor in the spleen beyond the capsule. It is well known that lymphatics are abundant in tendons; but they have not been demonstrated in striated muscle. On the other hand, it has been definitely shown, both in the embryo and in the adult, that there are no lymphatics in the central nervous system.

To this very general account of the lymphatic system in the mammal certain interesting facts from comparative anatomy must be added. It has long been known that there are pulsating lymph hearts in the amphibia. These lymph hearts arise as lymph sacs from the vertebral veins in the neck and from the coccygeal veins at the root of the tail. These sacs are close to the myotomes and develop striated muscle in their walls. In the birds there is a very interesting lymphatic system. There is a jugular lymphatic plexus which later becomes a lymphatic gland, and a caudal pulsating lymph heart, which develops from the coccygeal veins. In mammals the lymph sacs develop into groups of lymph glands, which may be called the primary glands for each region, while secondary glands develop along the lymphatic ducts.

In this brief résumé of the lymphatic system I have given only facts which can be clearly demonstrated. There are these sacs against the veins, and if injections are made from them one can demonstrate a gradually increasing plexus of vessels. These facts, however, but lead us on to seek their meaning. What are lymphatic capillaries, how do they arise, and how do they grow? There is general agreement that the lymphatics arise from certain centers and grow toward the periphery; but there are two theories as to how they grow. The theory which I hold is that the lymphatics arise from the endothelium of the veins and

grow by the multiplication of endothelial cells. The opposing theory holds that the lymphatics arise from tissue-spaces and grow by adding on new tissue-spaces; that beyond the tip of a definite completed vessel, which can be injected, are tissue-spaces which will be added to the capillary.

It is here necessary to submit the different types of method and the nature of the evidence which has been brought forth under the stimulus of these two theories. Some of the methods are direct, some indirect, but in all there is an effort to understand the nature of that very interesting and important tissue, the endothelial cell.

First, in regard to the nature of the earliest lymphatic buds, it is clear from sections, both of mammals and of birds, that these buds are lined by endothelium, but it proved very difficult to determine from sections that these buds were from the beginning connected with the veins. Eleanor Clark,[14] however, was able to test this point in the case of the lymphatics of the chick by developing a method for observing the tiny red buds in the living embryo. Into these lymphatic buds she injected a few granules of ink, and then observed the granules entering the vein. Moreover, in the amphibia Fedorowicz[15] has traced each step of the origin of the lymphatic buds from the veins, by specific differences between the endothelium and the mesenchyme.

From these early lymphatic buds it is possible to inject an increasing plexus of lymphatic capillaries as the embryo develops, and by this method to follow the lymphatic capillaries to their form in the adult, in the few places where that form is known. On this evidence was based the theory of the centrifugal invasion of the body by lymphatic capillaries. The next method of study which occupied the attention of the group of anatomists who were trying to follow the development of the lymphatic system was a comparison of the adequacy of the method in injection with the adequacy of the method of reconstruction of lymphatics from serial sections as applied to the problem of growth. This long series of studies followed an observation of Lewis[16] that if the lymphatics were reconstructed from sections they would appear as isolated vesicles for which no connections could be found. This is the experience of all who attempt to reconstruct an uninjected capillary plexus from sections, and therefore it has been necessary to test the limitations of the method. It is claimed that the method of reconstruction reveals more lymphatics than can be shown by the injection method, as it shows not only all the lymphatics which can be injected, but also the spaces that will be added to the plexus later. Moreover, it is on the evidence of reconstructions that the theory of the growth of lymphatics by the addition of tissue-spaces is based. It is true, of course, that injections would not fill up solid sprouts of endothelium, and everyone who has made injections of lymphatics is familiar with the difficulties of obtaining perfect specimens, but it has been demonstrated that when an area is chosen which can be adequately injected, more of a capillary plexus can be shown than can be reconstructed. For example, Eleanor Clark[17] has published a picture of an injection of the jugular lymphatic plexus of a chick which showed a far more extensive plexus than was demonstrated in a reconstruction of the same stage, previously recorded by Miller.[18] The two pictures, side by side,

[14] Clark, E. R. and E. L., *Anat. Record,* Vol. 6, 1912.

[15] Fedorowicz, S., *Bull. d. l'Acad. d. Sciences d. Cracovie,* 1913.

[16] Lewis, F. T., *Amer. Jour. of Anat.,* Vol. 5, 1906.

[17] Clark, Eleanor L., *Anat. Record,* Vol. 6, 1912.

[18] Miller, A. M., *Amer. Jour. of Anat.,* Vol. 12, 1912.

afford a striking contrast. The amount of the plexus which can be demonstrated by reconstruction increases very much if an oil immersion lens is used, but the method, though one of the most important aids in embryology, is entirely inadequate to test the method of growth of capillaries. No one would regard it as adequate to determine an entire plexus of blood capillaries even where their pattern is well known.

It is, I think, obvious that the only adequate method for the study of the growth of capillaries is to observe them in a living specimen; and in this connection we have a long series of valuable observations on the classical object, the living tadpole's tail. Capillaries were first seen in the tadpole's tail by Schwann, and were first differentiated into two types, blood-capillaries and lymphatic-capillaries, by Kölliker. During a long series of studies with this object, by Remak, Sigmund Meyer and others, and finally by Eliot R. Clark,[19] with greatly improved methods, two facts have become established—first, that endothelium is contractile and second that the vessels grow by the cell division of their own walls. Clark was able to watch a given lymphatic for several days and to observe that the wall put forth tiny processes of protoplasm, which we term sprouts, that the nuclei of the cell divided and wandered into the new sprouts, which developed into new vessels. He was able to plot out every mesenchymal cell in the neighborhood and to show that the growing sprouts of endothelium avoided rather than approached the processes of mesenchyme, and never incorporated them into their walls. Thus in the one place where natural conditions are such that every cell, or rather every nuclear area of a growing vessel, and every mesenchymal cell can be identified, it is without question true that both blood-capillaries and lymphatic capillaries grow through the proliferation of their own walls.

[19] Clark, E. R., *Anat. Record,* Vol. 3, 1909. *Amer. Jour. of Anat.,* Vol. 13, 1912. "Proc. Amer. Asso. of Anat.," *Anat. Record,* Vol. 8, 1914.

The method of growth of capillaries may thus be regarded as established. But this is not the whole problem for the embryologist. Under development he must consider both the original differentiation of tissues and their method of growth. In embryology it has become clear that there is a gradual differentiation of tissues from a common cell mass, and that after a tissue is once differentiated it increases by cell-division. This conception of the differentiation of tissues was clearly stated by von Baer in 1828. He called the process histological differentiation. Thus, development consists in the differentiation of tissues followed by growth. The most recent work on the lymphatic system demonstrates that the period of differentiation of endothelium is the period of the origin of the blood-vessels, and that this period has long since passed when lymphatics begin. Lymphatics do not differentiate from mesenchyme, but grow from veins.

It is well known that methods have long been sought by histologists to distinguish endothelium from mesenchyme. If we could always distinguish endothelium in sections the problem would be practically solved, but the difficulty of determining lymphatic endothelium in the sinuses of lymph glands, or vascular endothelium in the spleen pulp are too well known to need emphasis. These very difficulties lead us to the question, is endothelium differentiated from mesenchyme?

Efforts to distinguish endothelium from mesenchyme have not been entirely without results. For example, Clark has found that in the chick the nuclei of lymphatic endothelium can be distinguished from the nuclei of the mesenchyme by characteristic

nucleoli. Again Kampmeier[20] has shown that both venous and lymphatic endothelium in the toad can be distinguished from mesenchyme at certain stages by the presence of a greater number of yolk globules. Indeed, this differentiation of vascular and lymphatic endothelium from the mesenchyme was so striking as to convince Kampmeier that the lymphatics arose from the veins, though he had previously held the view that they arose from tissue-spaces.

These observations, valuable as they are, are not sufficiently universal to determine the nature of endothelium. The lymphatic endothelium grows from the endothelium of the veins; but since it varies slightly from the venous endothelium we may say that it is secondarily differentiated from it. This idea leads us directly to the most fundamental problem connected with the entire vascular system—namely, how does endothelium arise, how do the first endothelial cells differentiate? The question of the origin and the growth of the lymphatic system will not be completely solved until its essential tissue endothelium is completely understood. This leads us to seek for the origin of the first blood-vessels.

The question of the origin of the heart and blood-vessels has a vast literature. Since the time of Wolff and Pander, it has been known that blood-islands in the chick arise in the wall of the yolk sac. Then His[21] discovered that blood-vessels arise by a differentiation of vaso-formative cells or angioblasts. This is the fundamental point which recent work confirms, His having proved that angioblasts differentiated in the wall of the yolk-sac, and having seen that they did invade the embryo, advanced the hypothesis that all the angioblasts differentiated in the yolk-sac and then invaded the body from the embryonic membranes. The theory regarding angioblasts thus became centered around the idea of this invasion, and the more fundamental point was obscured. In recent years this theory that all of the vessels of the embryo are derived from the vessels of the membranes has been disproved by certain experiments of Hahn.[22] Hahn selected chicks in the stage of the primitive streak and burned out the membranes opposite the posterior end of the streak. In a few specimens which lived he found a small aorta and cardinal veins on the injured side of the embryo. These results have been confirmed by Miller and McWhorter[23] and by Reagan[24] on the chick and again by studies on the fish embryo by Stockard.[25] It may thus be regarded as proved that blood-vessels arise both within the embryo and in the embryonic membranes.

Stockard then went on to attack the more fundamental problem, how does endothelium first arise? In studies made on the yolk sac of the living fish embryo, he found that endothelium arises as spindle cells which differentiate out of mesenchyme. Moreover, he found that the endothelial cell was distinct from the blood-cell. This confirmation of the angioblast of His I regard as a very important contribution.

It is very clear in following the work of His, that he made studies on the living blastoderm of the chick, but so far as I am aware McWhorter and Whipple[26] were the first to study the living blastoderm of the chick in a hanging-drop preparation. By

[20] Kampmeier, O. F., *Amer. Jour. of Anat.*, Vol. 17, 1915.

[21] His, W., Untersuchungen über dir erste Anlage des Wirbelthierleibes, Leipzig, 1868.

[22] Hahn, H., Arch. f. Entwicklungsmechanik der Organismen, Bd. 27, 1909.

[23] Miller and McWhorter, *Anat. Record*, Vol. 8, 1914.

[24] Reagan, F. P., *Anat. Record*, Vol. 9, 1915.

[25] Stockard, C. R., *Amer. Jour. of Anat.*, Vol. 18, 1915. Two articles.

[26] McWhorter and Whipple, *Anat. Record*, Vol. 6, 1912.

using this method, I find, just as did His, that blood-vessels begin by a differentiation of cells. It is difficult to be sure of the first cells in the living chick which become angioblasts, but by the time the first cleft appears which indicates the position of the two upper myotomes there is an extensive plexus of bands of cells in the area vasculosa. In watching these bands of cells in the living specimen, I thought for some time that they could be differentiated by a slightly greater refractility than the rest of the tissue; but this did not prove to be an adequate criterion, for when the syncytium of mesenchyme forms in the later stages it makes a network of the tissue which is just as refractile. Moreover, in the study of the early vessels in the living blastoderm it is extremely difficult to tell which is the vessel and which the interspace. However, I found that the bands of endothelium or the definite vessels which form from them would suddenly change their appearance over wide areas, becoming intensely refractile and very granular and opaque. In this stage, which is so striking that it can be seen under low powers of the microscope, the vessels lose all appearance of being hollow; and I soon found that this was because every cell was passing into the phase of cell-division. This was proved by the rows of spindles in stained specimens.

The extent of cell-division in these chick embryos is most interesting. At times wide areas of the endoderm cell divide and become so opaque as to entirely obscure the cells beneath, and one has to wait until the endoderm becomes clear again. The difference in the reaction of the bands of endothelium and the syncytium of mesenchyme to cell division is a guide in the study of the early differentiation of blood-vessels. When the bands of endothelial cells divide the cells remain together: the outline of each cell becomes distinct, but they do not separate. In the case of the division of the cells of a syncytium of mesenchyme, however, many of the processes are withdrawn and the cell-body rounds up, so that it stands out as if it were an isolated cell, as has been described by Margaret Reed Lewis in tissue-cultures. Thus in areas in which it becomes very difficult to trace the ultimate strands of endothelium it may be necessary to wait for the phase of cell-division in one or the other tissue in order to make the distinction. In watching the vessels of the area vasculosa, one gets the suggestion that there may be a rhythm in cell-division. For example, if the area pellucida around the posterior end of the embryo be considered as divided into an inner and an outer zone, either all the vessels of the inner zone or all those of the outer zone may be found in cell division at the same time.

The vessels of the original plexus increase in size by cell division and new vessels are constantly formed within the plexus by numerous sprouts that grow out to connect its meshes. Beside this growth within the plexus there is an active differentiation of new endothelial cells, which can be watched in the living chick. In the early stages, up to five or six somites, there is no syncytium of mesenchyme and the wandering cells are scanty in number. Individual spindle-cells are thus clearly seen. They divide and at once show the essential characteristic of endothelium—that is, the tendency to form bands. Either an individual cell, or bands of two or three cells, send out tiny processes toward the older bands of endothelium, which at once respond by sending out tiny processes to meet the new ones. Thus endothelium consists of cells which differentiate as spindle-cells from the mesenchyme, and show at once two characteristics, first a tendency to remain

together after cell-division forming strands, and secondly, a tendency to join other bands of similar cells by protoplasmic processes. These bands of cells become blood vessels.

It is, I think, clear that the question now to be solved is how long does endothelium continue to differentiate out of mesenchyme? It can be seen to differentiate in the living chick in all the stages I have yet studied, that is in the stages before the circulation is established. This covers approximately the first two days of incubation. As is well known, there is a group of anatomists—Maximow, Reichert and Mollier, and a group of American workers, notably Huntington and McClure, who believe that endothelium continues to differentiate out of mesenchyme possibly throughout life. From the evidence which I have previously given I think it much more likely that endothelium will prove to have a limited period of differentiation, followed by growth. The study of the origin of blood-vessels seems to me to emphasize again the endothelial cell and to show that the vascular system arises from a differentiation, and growth of endothelial cells rather than by a dilatation of spaces.

In looking back over the history of the development of our knowledge of the lymphatic system, it is very clear that there have been periods of great activity followed by periods of rest. We are at present in a period of activity, and I should like to sum up what seem to me to be the results of the work of the last fifteen years. It has been shown that the problem of the origin of the lymphatic system is but a part of the general problem of the origin of the vascular system. Lymphatics are modified veins, in the sense that they grow from the veins. The veins are the primary absorbents and continue to take part in absorption throughout life. Up to the time of about six weeks for the human embryo, they are the only absorbents. Subsequently other systems develop, such as the arachnoidal villi and the lymphatic vessels, to assist in the function of absorption. The lymphatics only partially invade the body, and present indications point to the fact that their functions in absorption may be to some extent specific.

In an injection into the tissues of a dead organism it is essential to puncture the vessels of a plexus of lymphatic capillaries in order to fill lymphatics with a non-diffusible fluid. These injections demonstrate a complete wall, in the anatomical sense, which is ruptured only by increased pressure. In the living animal both true solutions and granules pass into lymphatic capillaries through the activities of endothelial cells or by means of wandering phagocytic cells.

This conception of the lymphatic system is at variance with the older idea of hazy lymphatic capillaries that faded off indefinitely through hypothetical lymph radicals into the tissue spaces. With the newer conception of definite lymphatic capillaries of endothelium it would be much better if we should revise the terms which developed in the period when our theories were vague and indefinite. In the first place there are "blood-capillaries," "lymphatic capillaries" and "tissue-spaces." If we should reserve the term "plasma" for the fluid within the blood-vessels, "lymph" for the fluid within the lymphatics and "tissue-fluid" for the fluid within the tissue-spaces, it would be a great gain in clearness. The term "tissue-fluid," meaning the fluid which is in the tissue-spaces of the living animal, should not be confused with the term "tissue-juice," by which the physiologist means the fluid which can be pressed out of the tissues. The term tissue-fluid should include such

special fluids as the cerebro-spinal fluid, the aqueous humor and the fluids of the serous cavities, as well as the general fluid of the less specialized tissue-spaces.

The study of the lymphatic system throws emphasis on the importance of tissue-spaces. I am convinced that the understanding of lymphatic capillaries as definite structures, definitely placed in restricted areas, forms a secure basis from which the varied problems of absorption may be solved.

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STATISTICAL PHYSICS[1]

Every physical measurement must be made in a region in equilibrium,[2] and nearly all of the correlations which have been established in physics, that is, nearly all physical laws, relate to substances in steady states or to substances in equilibrium. Furthermore, nearly all physical laws are one-to-one correspondences, and they are expressible as analytical functions. Thus the pressure of a given amount of a gas is an analytical function of the volume and temperature of the gas.

In every field of measurement, however, extreme refinement and care lead an investigator into a region of erratic action. This is evident when we consider that refined measurements are always subject to erratic error, and the atomic theory of the constitution of matter suggests that erratic action is always present everywhere, even in substances in complete thermal equilibrium.

It has long been the custom to speak of the probable error of a precise measurement *as if perfect precision would be possible if our measuring devices were perfect and free from erratic variations.* It is important, however, to recognize two distinct types of erratic error, namely, *extrinsic error* due to uncontrollable variability of the measuring device or system, and *intrinsic error* due to inherent variability of the thing or system which is being measured. Every physical measurement involves an operation of congruence, a standard of some kind is fitted to or made congruent with successive parts (which parts are thereby judged to be equal parts) of the thing or system which is being measured; and the standard system and the measured system are both subject to erratic variations.

There is, perhaps, no case in which intrinsic error and extrinsic error can be clearly distinguished and separated from each other; but when the errors of one kind are much larger than the errors of the other kind they can, of course, be recognized. It is proper to speak of the *probable error* of a single measurement when the variations of the measuring device or system are dominant, but one should speak of the *probable departure* of the measured system from a certain mean condition at any time when the "errors" of observation are due chiefly to variability of the thing or system which is being measured. Thus in measuring the coefficient of sliding friction extrinsic error may be made negligible by making the measurements carefully, but very large "errors" persist. The thing which is being measured is inherently indefinite, and it may at any time depart widely from its average value.[3] In measuring the loss of

[1] The substance of a lecture delivered by W. S. Franklin before the Department of Terrestrial Magnetism of the Carnegie Institution, Washington, D. C., December 20, 1915.

[2] Thermal equilibrium is here referred to; certain quasi states of thermal equilibrium being included. The only exception is the kind of measurement which consists of simple counting, like the counting of cattle as they pass through a gate or the counting of electrons as they enter an ionization chamber.

[3] A very brief but comprehensive statement of the proper precision method for the study of an erratic thing like friction is given by W. S. Franklin, *Transactions of American Institute of Electrical Engineers*, Vol. 20, pp. 285–286.